#25 Declaration
Evelyn 09/08/98

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of	:	Yi Li and Steven M. Ruben	
Serial No.	:	08/466,343	Group: 1804
Filed	:	June 6, 1995	Examiner: Basham, D.
For	:	HUMAN G-PROTEIN CHEMOKINE RECEPTOR HDGNR10	
Docket No.	:	325800-449 (PF189)	

Assistant Commissioner for Patents
Box Non-Final - Non Fee
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

SIR:

I, Steven M. Ruben (appended as Exhibit A is my *curriculum vitae*), as a co-inventor in the above-referenced application, do declare as follows:

1. I am familiar with the contents of the above-captioned application, including the communications issued to and from the Examiner. Thus, I am aware of the fact that the Examiner for the above-captioned application has questioned whether the G-protein chemokine receptor as set forth in Figure 1, whose sequence is set forth in the Sequence Listing as SEQ ID NO:2, has a practical use as a receptor for a ligand as described in the specification.

2. In particular, I am aware that, *inter alia*, on page 11, at lines 1-16, the originally filed specification of the above-captioned application describes the G-protein chemokine receptor as being capable of binding a ligand or ligands.

3. Also, I am aware that, *inter alia*, on page 3, in the last 10 lines of the page, the above-captioned application states that many chemokines and their receptors (including chemokine receptors similar to the chemokine receptor of Figure 1 (SEQ ID NO:2)) have pro-inflammatory activity and are involved in multiple steps during an inflammatory reaction.

4. Further, I am aware that the G-protein chemokine receptor shown in Figure 1 (whose amino acid sequence is SEQ ID NO:2 in the

Sequence Listing) is described in the paragraph bridging pages 6 and 7 of the specification, as being derived from human monocytes and having at least 70.1% identity to the chemokine receptor (see Figure 2) that binds to the human monocyte chemotractant protein (MCP)-1 ligand (a C-C cytokine ligand, see paragraph 5, below).

5. It was clearly recognized in this field prior to filing of the above-captioned application that the family of cytokine ligands having four spatially conserved cysteines, including two adjacent cysteines (C-C cytokines) (ligands sometimes called intercrines or chemokines) may have significant homology and can cross-compete for binding to common chemokine receptors. It was further recognized that multiple chemokine receptors having structural similarity can cross-compete for the same ligand(s). A example of such awareness of ligand cross-competition and receptor cross-competition in this field is set forth in the abstract and in column 1, page 360, of the article appended as Exhibit 1 (J. Mol. Imm. 30:359-367 (1993)).

6. Others in this field had observed prior to filing of the above-captioned application that cytokine receptors (such as the G-protein chemokine receptor of SEQ ID NO:2 (Figure 1)) that bind to one ligand member of the C-C cytokine family can often bind to other members of their family. In fact, it was known that a receptor member of the C-C cytokine family (C-C CKR1) would bind, *inter alia*, to three human ligands of the C-C cytokine family. The ligands are monocyte chemotactic protein (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and macrophage inflammatory protein-1ß (MIP-1ß). An example of such binding tendencies in this field is set forth in the abstract of the appended article which is marked as Exhibit 2 (Cell, 72:415-425 (1993)).

7. I further declare that binding assays for ligands to the polypeptide G-protein chemokine receptor having an amino acid sequence as set forth in SEQ ID NO:2 (see Figure 1) have been performed with positive results. Ligands do in fact exist that bind to such receptor and some such bindings have been published in the literature after the present application was filed. Such ligands that bind include C-C cytokine ligands which are well-known to be involved in an inflammatory response. In fact, the human ligands known as (MIP)-1α and (MIP)-1ß are examples of ligands that bind to the G-protein chemokine receptor set forth in Figure 1 (SEQ ID NO:2). Such binding of ligands (and other ligands) to the above-described receptor is readily verified by procedures set forth in the above-captioned application and, more particularly, by utilizing techniques well-known and routine in this field.

8. Utilizing a detectable form of the receptor (see page 27, lines 19-30, of the specification) and investigating the binding of such receptor to ligands involved in an inflammatory response as referred to on page 3, line 12, through page 4, line 15 of the specification (see paragraphs 2 and 3, above), including known ligands involved in the inflammatory response that are recognized in this field as being members of the C-C chemokine family (see paragraphs 5 and 6, above), readily results in binding of either MIP-1α or MIP-1ß to the receptor.

9. Once such ligand is bound as described in paragraph 8, above, and the bound receptor/ligand complex is detected, the complex is readily isolated using methods well-known in the art. The ligand may then be separated from the receptor/ligand complex and labelled to permit further investigation of receptor and ligand binding (see paragraph bridging pages 21-22 of specification). Utilizing the labelled ligand obtained as described in paragraph 8, above, the effects of receptor analogs, ligand analogs, antagonists or agonists on the receptor/ligand binding may be investigated by routine and well-known procedures. Ligand assay procedures are well-known to those in the chemokine receptor/ligand field and are described generally at page 27, lines 19-30, of the specification. Page 27, at lines 28-30, cross-references systems to assay for agonists and/or antagonists to the binding of such receptor to the ligand (such as receptor analogs that also bind the ligand). Such systems are also well-known in this field.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that the statements were made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under Sec. 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patents issued thereupon.

Date: 12/19/97

By: _____
Steven M. Ruben

N:\h\hgs-132.449

CURRICULUM VITAE

STEVEN M. RUBEN

Address: 18528 Heritage Hills Drive
Olney, MD 20832
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Date and Place of Birth: April 20, 1956, Cleveland, Ohio

Social Security No.: 274-42-1919

Marital Status: Married, 2 children

Business Address: Human Genome Sciences
9410 Key West Avenue
Rockville, MD 20850
(301) 251-6022

Education:

1988	Ph.D., Cell/Molecular Biology Program, University of Cincinnati, Cincinnati, OH Ralph R. Meyer, Ph.D. Advisor, Suppression of the *Escherichia coli ssb-1* mutation by an allele of *groEL*
1985	M.S., Cell/Molecular Biology Program, University of Cincinnati, Cincinnati, OH
1979	B.S., Biochemistry, Ohio State University, Columbus, OH

Honors & Awards:

1989-1992	Leukemia Society of America Fellowship
1985-1987	University Research Council Summer Fellowship, University of Cincinnati
1986	Outstanding Research Award, American Society for Microbiology, Ohio Branch
1982,1983	Harry L. Weiman Foundation Summer Fellowship, Department of Biological Sciences, University of Cincinnati
1980-1987	Graduate Teaching Assistantship, Department of Biological Sciences, University of Cincinnati
1978	Distinguished Achievement in Biological Sciences, Ohio State University

Professional Experience:

1996-Present	Director, Protein Therapeutic Department, Human Genome Sciences, Rockville, MD
1993-1996	Associate Director, Molecular Biology Department, Human Genome Sciences, Rockville, MD
1992-1993	Scientist, Molecular Biology Department, Human Genome Sciences, Rockville, MD
1991-1992	Research Associate, Laboratory of Dr. Craig Rosen Department of Gene Regulation, Roche Institute of Molecular Biology, Nutley, NJ
1989-Date	Leukemia Society of America Fellow, Laboratory of Dr. Craig Rosen Department of Gene Regulation, Roche Institute of Molecular Biology, Nutley, NJ and Department of Microbiology, University of Medicine and Dentistry of New Jersey, Newark, NJ

1987-1989 Postdoctoral Fellow, Laboratory of Dr. Craig Rosen, Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ

1980-1986 Teaching Assistant, General Biology Laboratory, Department of Biological Sciences, University of Cincinnati, Cincinnati, OH

Research Experience:

Director:

As director, I am responsible for eleven Ph.D. scientists and support staff. Major efforts include the secretory signal identification project. Aspects of this project include development of methodology for high throughput cloning, establishment of expression patterns using gridding arrays, establishment of methods for stable and transient expression of secreted candidates and development of assay systems to identify biological activities of these molecules.

Associate Director:

As an associate director, I was responsible for seven Ph.D. scientists and support staff. The department focused on therapeutic protein identification and biological characterization of candidate genes. As associate director, I coordinated the HGS cDNA library construction and sequencing schedules and pursued collaborations for novel tissue sources. In addition, I worked closely with the bioinformatics department to design and implement new strategies for candidate gene identification, including the strategies for secretory signal identification.

Scientist:

As a research scientist at HGS, my work initially focused on identification and characterization of novel therapeutic candidates from the HGS EST database. These candidates include a novel FAS ligand, a heart and lung specific DNase, and Stat6. In addition, work involved development of high throughput template production using a PCR approach which was incorporated into the HGS template standard operating procedure.

Postdoctoral research:

As a research associate my work has focused on the role that the *rel* family of proteins plays in the pathogenesis of both HIV-1 and HTLV-I. This family of proteins may play a critical role in activation of latent HIV virus. In addition, activation of these proteins by the HTLV-I Tax protein may play an important role in the pathways which lead to Adult T-cell Leukemia following HTLV-I infection. Using degenerate oligonucleotides corresponding to highly conserved regions of the *rel* proteins as primers in PCR reactions using various cDNA's as template, three new *rel*-related proteins were identified, including the p65 subunit of NF-kB. Another one of these genes, I-Rel, is an inhibitor of NF-kB function. These genes have been expressed for both mammalian expression and purification from *E. coli* and the transforming potential of these genes has been measured using various assays. In addition, I directed a summer student and am directing the research of a post-doctoral fellow aimed at structure-function analysis of these proteins.

Prior postdoctoral research has involved examination of the mechanisms of action of the viral regulatory proteins of HTLV-I and HIV-1 at both the nucleic acid and protein levels. This work emphasized the use of recombinant techniques of molecular biology as well as immunological analysis of protein expression in both eukaryotic and prokaryotic systems. Tissue culture work including transient and stable expression of viral genes was an integral part of this work. This work involved the construction and screening of cDNA libraries and isolation of monoclonal antibodies to study differential expression of cellular factors resulting from expression of viral proteins. The mechanism of action of the HIV-1 Tat protein was also studied at both the level of interaction with the RNA target sequence, Tar and direct protein-protein associations with a family of host proteins thought to be involved in regulation of transcription.

Graduate research:

My thesis research emphasized the use of molecular biological tools in studying protein-protein interactions of an *E. coli* DNA binding protein (SSB). This work resulted in identification of the initial *in vivo* interactions involving SSB and the heat shock protein Gro EL. In addition to the standard recombinant DNA methodology, this work involved the construction and screening of genomic libraries, protein purification, bacterial genetics, immunological techniques including Western blotting and RIA, and both *in vivo* and *in vitro* DNA synthesis studies.

Societies:

American Society for the Advancement of Science
American Society for Microbiology

Publications:

1. Meyer, R.R., Voegele, D.W., Ruben, S.M., Rein, D.C. and Trela, J.M. (1982). Influence of single-strand DNA-binding protein on *Rec*A induction in *Escherichia coli*. Mutat. Res. 94, 299. _

2. Ruben, S.M., Van Den Brink, S.W., Rein, D.C. and Meyer, R.R. (1985). Protein interactions of single-stranded DNA-binding protein determined by second-site revertent analysis. Ohio J. of Science 85, 62.

3. Meyer, R.M., Ruben, S.M., Van Den Brink-Webb, S.E., Laine, P.S., Perrino, F.W. and Rein, D.C. (1988). Protein-protein interactions of *Escherichia coli* single-stranded DNA-binding protein. In *DNA Replication and Mutagenesis*, R.E. Moses and W.C. Summers, eds., American Society for Microbiology, Washington, DC, pp. 154-162.

4. Ruben, S.M., Van Den Brink-Webb, S.E., Rein, D.C., and Meyer, R.R. (1991). Suppression of *ssb*-1 thermosensitive allele by GroEL mutant protein in *Escherichia coli*: effects on phage lambda growth. (submitted).

5. Ruben, S.M., Van Den Brink-Webb, S.E., Rein, D.C. and Meyer, R.R. (1988). Identification of an *in vivo* interaction between *ssb* and GroEL protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 85, 3767.

6. Ruben, S., Poteat, H., Tan, T.-H, Kawakami, K., Roeder, R., Haseltine, W. and Rosen, C.A. (1988). Identification of a cellular transcription factor required for regulation of interleukin-2 receptor gene expression by human T-cell leukemia virus *tat* gene product. Science 241, 89-91.

7. Ruben, S., Perkins, A., Purcell, R., Joung, K., Sia, R., Burghoff, R., Haseltine, W.A. and Rosen, C.A. (1989). Structural and functional characterization of the human immunodeficiency virus *tat* protein J. Virol. 63, 1-8.

8. Perkins, A., Cochrane, A., Ruben, S. and Rosen, C.A. (1989). Structural and functional characterization of the human immunodeficiency virus *rev* protein. J. Acquir. Imm. Def. Syn. 2, 256-267.

9. Cochrane, A., Kramer, R., Ruben, S., Levine, J. and Rosen, C.A. (1989). The human immunodeficiency virus *rev* protein is a nuclear phosphoprotein. Virology 171, 264-266.

10. Cochrane, A.W., Golob, E., Volsky, D., Ruben, S., Rosen, C.A. (1989) Functional significance of phosphorylation to human immunodeficiency virus *rev* protein. J. Virol. 63, 4438-4440.

11. Cochrane, A., Ruben, S., Nelbock, P. and Rosen, C. A. (1989). Functional and structural domains of the human immunodeficiency virus transacting regulatory proteins *tat* and *rev* in *Human Retroviruses.* Vol. 119, Alan Liss, Inc..

12. Ruben, S.M., Perkins, A., and Rosen, C.A. (1989) Activation of NFkB by the HTLV-I trans-activating protein requires an additional factor present in lymphoid cells. New Biologist 1, 275-284.

13. Kramer, R.A., Tomchak, L., Ruben S., and Rosen, C.A. (1990) Yeast cells expressing the *tax* gene of Human T-cell Leukemia Virus Type I exhibit a Flocculaction phenotype identical to *FLO1* mutants. Aids Research and Human Retroviruses 6, 1305-1309

14. Ruben, S.M. and Rosen, C.A. (1990). Constitutive expression of the HTLV-I Tax protein suppresses signals required for activation of the transcription factor, NFkB. New Biologist 2, 894-902

15. Rosen, C. A. and Ruben, S. M. (1991), Regulation of Human Retroviruses. Drug News and Perspectives, 4, 340-351.

16. Ruben, S.M., Dillon,P.J, Schreck, R., Henkel, T.,Chen,C-H, Maher,M. Baeuerle P.A., and Rosen,C.A. (1991) Isolation of a *rel* related human cDNA that potentially encodes the 65kD subunit of NFkB. Science 251, 1490-1493.

17. Rosen, C.A., and Ruben, S.M., Function of human retrovirus regulatory proteins. (1991) in Annual Review of Medicinal Chemistry, ed. C. Plattner, 171-180.

18. Ruben, S.M., Narayanan,R., Klement, J.F., and Rosen,C.A., (1992) An alternatively spliced form of NF-kB p65 defines an essential functional domain, Mol Cell. Biol.12, 244-255.

19. Ruben, S.M. Klement, J.F., Maher, M., Coleman, T.A., Chen, C.-H.,and Rosen, C.A. (1992) I-Rel: A rel-related protein that inhibits NF-kB function, Genes Develop, 6,745-760.

20 Narayanan, R., Klement, J.F., Ruben, S.M., Higgins, K., and Rosen, C.A., (1992) Identification of a naturally occuring transforming varient of the p65 subunit of NF-kB, Science 256,367-370.

21. Kunsch, C., Ruben, S.M., and Rosen, C.A. (1992) Selection of optimal kB/Rel DNA binding motifs: Interaction of both subunits of NF-kB with DNA is required for transcriptional activation, Mol. Cell. Biol. 12,4412-4421.

22. Rosen, C.A., Dillon, P.J., Olsen, H.S., and Ruben, S.M., (1992) Complexities of Human Retrovirus Gene Expression, p.235-254 in *Genome Research in Molecular Medicine and Virology*., Academic Press, Inc., Orlando, FL, ed. K.W. Adolf.

23. Ruben, S.M., Beg, A.A., Scheinman, R.S., Haskill, S., Rosen, C.A., and Baldwin, Jr. A.S., (1992) IkB/MAD-3 targets the nuclear localization sequences of c-Rel and of the p50 and p65 subunits of NF-kB, Genes & Develop. 6, 1899-1913.

24. Moore, P., Ruben, S.M., and Rosen, C.A., (1993) Conservation of transcriptional activation functions of the NF-kB p50 and p65 subunits in mammalian cells and *Saccharomyces cerevisiae.* Mol. Cell. Biol. 13, 1666-1674.

25 Ohana, B., Moore, P., Ruben, S.M., Southgate, C.D., Green, M., and Rosen, C.A. (1993) The HIV-1 Tat binding protein is a transcriptional activator belonging to a new family of evolutionarily conserved genes, Proc. Natl. Acad. Sci. 90, 138-142

26. Coleman, T.A., Kunsch, C., Maher, M., Ruben, S.M., and Rosen, C.A. (1993) Acquisition of NFKB1-selective DNA-binding by substitution of four amino acid residues from NFκB1 into RelA. Mol. Cell. Biol. 13, 3850-3859.

27. Papadopoulos, N., Nicolaides, N.C., Wei, Y.F., Ruben, S.M., Carter, K.C., Rosen, C.A., Haseltine, W.H., Fleischmann, R.D., Fraser, C.M., Adams, M.D., Venter, J.C., Hamilton, S.R., Petersen, G.M., Watson, P., Lynch, H.T., Peltomaki, P., Mecklin, J.P., Chapelle, A.D., Kinzler, K.W. & Vogelstein, B. (1994) Mutation of a mutL homolog in hereditary colon cancer. Science 263, 1625-1629.

28. Nicolaides, N.C., Papadopoulos, N., Liu, B., Wei, Y.-F., Carter, K.C.,Ruben, S.M., Rosen, C.A., Haseltine, W.H., Fleischmann, R.D., Fraser, C.M., Adams, M.D., Venter, J.C., Dunlop, M.G., Hamilton, S.R., Petersen, G.M., Chapelle, A.D., Vogelstein, B. and Kinzler, K.W. (1994). Mutations of two PMS homologues in hereditary nonpolyposis colon cancer, Nature 371, 75-80.

29. Adams, M.D., Kerlavage, A.R., Fleischmann, R.D., Fuldner, R.A., Bult, C.J., Lee, N.H., Kirkness, E.F., Weinstock, K.G., Gocayne, J.D., White, O., Suttton, G., Blake, J.A., Brandon, R.C., Chiu, M-W., Clayton, R.A., Cline, R.T., et al. (1995). Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. Nature 377:3.

30. Quelle, F.W., Shimoda, K., Thierfelder, W., Fischer, C., Kim, A., Ruben, S.M., Cleveland, J.L., Pierce, J.H., Keegan, A.D., Nelms, K., et al. (1995). Cloning of murine Stat6 and human Stat5, Stat proteins that are tyrosine phosphorylated in responses to IL-4 and IL-3 but are not required for mitogenesis. Mol Cell Biol 15(6):3336-43.

31. Coleman, T.A., Huddleston, K.A., Ruben, S.M., Rosen, C.A., and Gentz, R. (1997) Expression and Reconstitution of NF-κB from Insect Cells Using a Baculovirus Vector. Protein Expression and Purification 9, 40-48.

32. Zeng, Z., Parmelee, D., Hyaw, H., Coleman, T.A., Su, K., Zhang, J., Gentz, R., Ruben, S., Rosen, C. and Li, Y. (1997). Cloning and Characterization of a Novel Human DNase. Biochemical and Biophysical Research Communications 231, 499-504.

33. Hu, J-S., Hastings, G.A., Cherry, S., Gentz, R., Ruben, S., and Coleman, T.A. (1997) A novel regulatory function of cleaved VEGF-2 for vascular endothelial and smooth muscle cells. (Submitted).

Recent Presentations

1. "Use of high-throughput analysis of sequences for the discovery of novel therapeutics and diagnostics" Impact of Genomics on Inflammation Research, New York Academy of Sciences, October 18, 1995

2. "Use of high-throughput analysis of sequences for the discovery of novel therapeutics and diagnostics" Genome-Based Drug Discovery, International Business Communications, March 21-22, 1996.

3. "Utilizing High Throughput Sequencing for the discovery of Novel Therapeutics and Diagnostic Targets" The Application of Genomics to Drug Discovery, Strategic Research Institute, May 30-31, 1996.

Patents

Granted

1. PCT 5,556,767 Issued On 17-Sep-96, filed 12/22/93, Macrophage Inflammatory Protein Gamma.

2. PCT 5,504,003 Issued On 02-Apr-96, filed 3/8/94, Human Chemokine Beta-8, Chemokine Beta-1 and Macrophage.

3. Filed 5/5/94, Human DNase .

4. Filed 1/26/95, HumanDNA Mismatch Repair Protein.

Pending

39 patents pending.

Published

15 patents published.